NOTES ON A FATAL EPIDEMIC INTESTINAL DISEASE OF GOLDFISH.

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(Plate II.)

The epidemic herein described occurred amongst "Comet," "Fantail," and "Calico" Goldfish in the smaller aquaria of Mr. J. C. Brunnich, Agricultural Chemist. All developmental stages fell a prey to the disease. The tanks were well aerated, and had been in use for a long time, but it was only in those tanks where hard tap-water had been used to replenish the supply that the fish contracted the disease; whilst in the larger outdoor ponds and tanks no infection seems to have taken place. Food consisting of white worms (Enchytræ) reared in special containers; scraped meat and artificial foods were given alternately. To understand the problem thoroughly, a further and more detailed examination of the actual waters involved is imperative.

The aquarist is well aware that sudden fatal epidemics among fish are liable to occur at any time and without apparent cause. Sometimes deaths can be definitely traced to fungoid diseases, but more often than not the evil is deep-seated and is due to some other cause as yet only surmised. From time to time such epidemics have been noted, but in most cases death intervenes before any preventive measures can be adopted, and sometimes all the inhabitants of an apparently healthy aquarium die before the seriousness of the outbreak can be realised.

In aquaria directly under my care, containing local fish and a few "tropicals," similar experiences have been frequent, and for some years now an opportunity has been sought of becoming better acquainted with fish diseases so as to enable a diagnosis to be made sufficiently early, to save considerable mortality, always so characteristic of such epidemics.

In this particular case the symptoms are quite definite, so that it should be possible to detect the disease in the early stages of development. It is interesting in the first place to notice that there appears to be a seasonal appearance of such epidemics. I make this statement guardedly because as yet we have very little idea as to the cause of the seasonal occurrence, and consequently because the disease seems to break out quite independently, in different adjoining aquaria at the same time almost to a day, it is seen to be very definite in its action and very deadly, the source of the infection remaining as much as ever a mystery.

Superficially the water in the aquaria gives little indication of impending tragedy, and it is not until the fish begin to suffer disintegration of the abdominal contents that the abnormality becomes distinctly noticeable, the fish resting motionless on the bottom of the tank. Now and again, as if to cast off the discomfort occasioned thereby, a fish will make a sudden dart through the water only to come to rest again with extreme suddenness. This action seems to denote a final stage in the development of the disease.

It is I think recognised that it is desirable from time to time to replace the chemicals in the water which are used up during the healthy life of the fish, and small quantities of magnesium sulphate and sodium chloride materially assist towards this end, whilst the addition of plaster of paris provides for a possibility of calcium deficiency. In this instance the use of plaster of paris arrested the disease.

The readiness with which the $CaSO_4$ is dissolved in "spent" aquaria containing an undue accumulation of carbon dioxide as the result of the presence of putrefying substances in the water, followed by a period of rapid oxygen consumption, suggests the necessity of such chemicals. Soon after the calcium sulphate is added to the water, precipitation takes place and the water, becoming wonderfully clear, provides an improved environment for the fish themselves, which soon react to the changed conditions. As soon as an equilibrium has been reached, the $CaSO_4$ may be removed, but by this time a higher alkalinity has been reached. Now although our larvivorous fish will live in slight acidity, say pH 6·0, nevertheless it is a matter of frequent comment that they do so only under protest, and that they seem more prone to disease than they do in alkaline waters. The acidity of the water seems to synchronise also with the slow but sure destruction of the snails; *Bullinus pectorosus* Conrad and *Limnæa lessoni* Desh. do not seem to thrive in such a medium.—R.H.H.

Pathology of the Disease.

Three fish were examined post-mortem. The pathological findings resembled very closely those found in cholera in the human; they were as follow:—

There was constantly a deep bile-tinged staining under the skin of the abdomen just ventral and caudal to the left pectoral fin. One specimen showed necrosis of the body wall at the site of the staining, the skin being thinned, of the texture of tissue paper, and was about to slough. There were no other external features of interest. On opening the body, the respiratory system I found to be, as far as I could judge, normal.

On examination of the abdominal contents, I found practically the whole of the intestines involved in a gangrenous process, only about a centimetre of the terminal portion escaping. The bile channels were also involved, leading to rupture and consequent staining of the abdominal wall, as noted above. I could not judge macroscopically whether the liver was involved. In two specimens, the necrotic process had spread to the swim bladder, which no

doubt led to the condition observed by my co-author, namely, falling to the bottom of the tank and inability to rise to the surface of the water.

Histology of the Disease.

This may best be described in stages illustrated by the figures (Plate II.).

Intestine.—First stage: Acute inflammatory exudate in the villi and submucosa (Figure 2, left, and Figure 3).

Second stage: Sloughing of the mucosa but epithelial cells staining well (Figure 4).

Third stage: Sloughing with marked cellular degeneration of epithelium. General structure of the gut is fairly well maintained (Figure 5).

Fourth stage: Necrosis amd sloughing of the whole villi, musculature still intact (Figure 6).

Fifth stage: Complete necrosis of the whole intestinal wall. Amorphous sloughs in the lumen (Figure 7).

Liver.—This organ showed complete necrosis, the nuclei of the cells having completely degenerated while their cytoplasm stained very feebly with cytoplasmic dyes. There was no evidence of primary fatty degeneration so I judge the intoxication to have been of an overwhelming kind, similar to acute necrosis in the human. The general texture of the viscus was unrecognisable.

Etiology.

The three fish that form the subject of the above comment were submitted to me in formalin. Successful cultures of the intestinal flora could not then be anticipated. Direct smears showed as the only significant feature the presence of a subterminal spored clostridium.

I had the opportunity of culturing the gut contents of another fish which had died of the same disease. Direct smears showed an unusual organism in my experience of intestinal flora, namely, a small non-motile Gram-negative diplobacillus in almost all cases encapsuled. Very rarely what appeared to be the same organism was single and not encapsuled. Variation in size was considerable, from 1·25 to 4 microns averaging about 2 microns in length by 0·5 micron in width. An emulsion of gut content was plated out on McConkey's medium. The organism evidently fermented lactose as no pale colonies showed up. Examination showed the diplobacillus still present, and to obtain a pure culture a subculture was made on a plain agar plate. No growth of the germ desired was obtained, or at least it was overgrown to such an extent that it could not be recovered. A similar fate befell those on the first plate, and the organism, evidently very delicate, was lost. I do not suggest this bacillus is the cause of the disease, but an organism of the kind noted seemed to me so unusual that I judged it wise to follow it up, and I mention it here for the information of workers in this field.

An interesting point in the epidemiology of the disease was put to me by Mr. Marshall, of the Queensland Museum scientific staff. He desired to know why fish, which were transported all the way from Japan in the same water unchanged and therefore likely to be foul and heavily infected and survived this apparently unfavourable medium, died, as did many recently imported fish, when taken ashore and put in clean water of a composition assumed by experienced aquarists to be a suitable environment. The only explanation that I can offer is that the original tanks in which the fish were transported contained bacteriophage in the water, as might very easily happen. In the event of another epidemic of this kind I propose to test this hypothesis experimentally.

The above work is necessarily only preliminary, since the amount of material available was very scanty, and was done without access to much literature on the subject.

The Medical Research Council[1] make a small passing reference to the fact that a cholera-like disease occurs in fish.

T. P. Hughes[2] reports an exhaustive investigation of fowl cholera, and describes as constantly occurring a "small pleomorphic, bipolar staining, Gram-negative, non-motile bacillus," which rather resembles that which I have described in this instance. I read Hughes's paper after I suspected this bacillus as having some causal relationship with the disease, and now feel that more material treated by more refined methods may enable me to solve the problem of etiology.—J.V.D.

[1] Med. Res. Council : "A System of Bacteriology," 1929, vol. iv, p. 436.

[2] Hughes, T. P.: Jl. of Exptl. Medicine, 1930, 51, 225.